

Severe intrauterine growth retardation with increased mitomycin C sensitivity: a further chromosome breakage syndrome

C G Woods, M Leversha, J G Rogers

Abstract

We report an infant with pre- and postnatal microcephaly and growth retardation, a distinctive face, and developmental delay. The initial diagnosis was of Seckel syndrome. He became pancytopenic at 16 months and died soon after. His bone marrow was of normal cellularity but had a small lymphocyte infiltration. Increased spontaneous chromosome breakage was seen in blood and fibroblasts. Mitomycin C induced chromosome damage was increased and comparable to that seen in Fanconi anaemia. Reports of similar patients are reviewed. This entity of severe intrauterine growth retardation and increased mitomycin C sensitivity is hypothesised to be a distinct chromosome breakage syndrome.

(J Med Genet 1995;32:301-305)

Seckel syndrome is a rare autosomal recessive phenotype of unknown aetiology and heterogeneity. The principal features are severe pre- and postnatal growth retardation, severe pre- and postnatal microcephaly, mental retardation, and a characteristic face with a prominent midface, lobeless ears, and a beaked, apparently large, nose.¹ Cleft palate and elbow anomalies are also reported to occur frequently.² The condition has probably been over diagnosed and it is now thought likely that only one of the two cases personally reported by Seckel had the disorder.^{1,3,4}

Over 50 genes are known to be involved in DNA repair in yeast.⁵ Given the considerable homology with mammalian systems, at least that number would be expected to exist in man.⁶ The number of known human DNA repair genes exceeds the number of known phenotypes, for example, the "chromosome breakage syndromes" such as ataxia telangiectasia, Fanconi anaemia, and Bloom syndrome. Characteristic shared features of chromosome breakage syndromes are small size at birth and, thereafter, an increased incidence of malignancies and pancytopenia and increased spontaneous and induced chromosome damage. These features are present in the child we report and some previously documented cases of possible Seckel syndrome. The absence of specific features present in the known chromosome breakage syndrome allows these to be eliminated as possible diagnoses. This case report supports the hypothesis that one of the

severe growth retardation phenotypes involves a defect of DNA repair.

Case report

A male infant was born at 37 weeks of gestation by vaginal delivery after an apparently uneventful pregnancy. He was the second child of non-consanguineous parents. They had had four previous first trimester miscarriages of unknown cause and a healthy daughter. The father (Yugoslavian) was 31 years old and the mother (Albanian) was 26 years old at the time of birth. Birth weight was 1500 g, length 40 cm, and head circumference 26 cm (all well below the 3rd centile). The Apgar scores were 9 at one minute and 10 at five minutes. On examination there was obvious microcephaly and growth retardation, micrognathia, sloping forehead, and a prominent midface with a slightly hooked nose (fig 1A and B). A provisional diagnosis of Seckel syndrome was made.

The following tests were normal: urinary metabolic screen, serum phenylalanine, a TORCH screen performed at 1 week of age, and a skull x ray. A cerebral CT scan showed very small ventricles but no other abnormalities. At 3 weeks of age a skeletal survey showed osteopenia with thin, gracile long bones but no abnormalities of the hand, hip, ribs, radius, or ulnar bones.

At 5 months he had an episode of choking and cyanosis owing to gastro-oesophageal reflux. At 9 months grand mal fits began which were controlled with carbamazepine. Chromosome analysis at 9 months showed an increased level of spontaneous chromosome breakage.

At the age of 16 months his weight was 4.45 kg, length 67 cm, and head circumference 34 cm (all well below the 3rd centile). He had obvious microcephaly, apparent hypertelorism, the midface and bridge of nose were prominent, and the nose appeared beaked with a hypoplastic columella. The ears had a simple helical pattern with absence of the lobes. The face showed little resemblance to Seckel syndrome (fig 1C). The testes were undescended and there was scrotal hypoplasia and glandular hypospadias. Multiple small patches of hyper- and hypopigmentation which were not distributed in the lines of Blaschko were seen predominantly over the abdomen (fig 1D). The fingers were spindle shaped, the first toes were cocked up and slightly laterally deviated, and both ankles were plantar flexed. The child was

The Murdoch Institute for Research into Birth Defects, 10th Floor, Royal Children's Hospital, Flemington Road, Parkville 3052, Australia
C G Woods
J G Rogers

Sanger Centre, Hinxton Hall, Hinxton, Cambridge CB10 1RQ, UK
M Leversha

Correspondence to:
Dr Woods, Department of Clinical Genetics, St James's University Hospital, Beckett Street, Leeds LS9 7TF, UK.

Received 11 October 1994
Revised version accepted for publication 29 November 1994

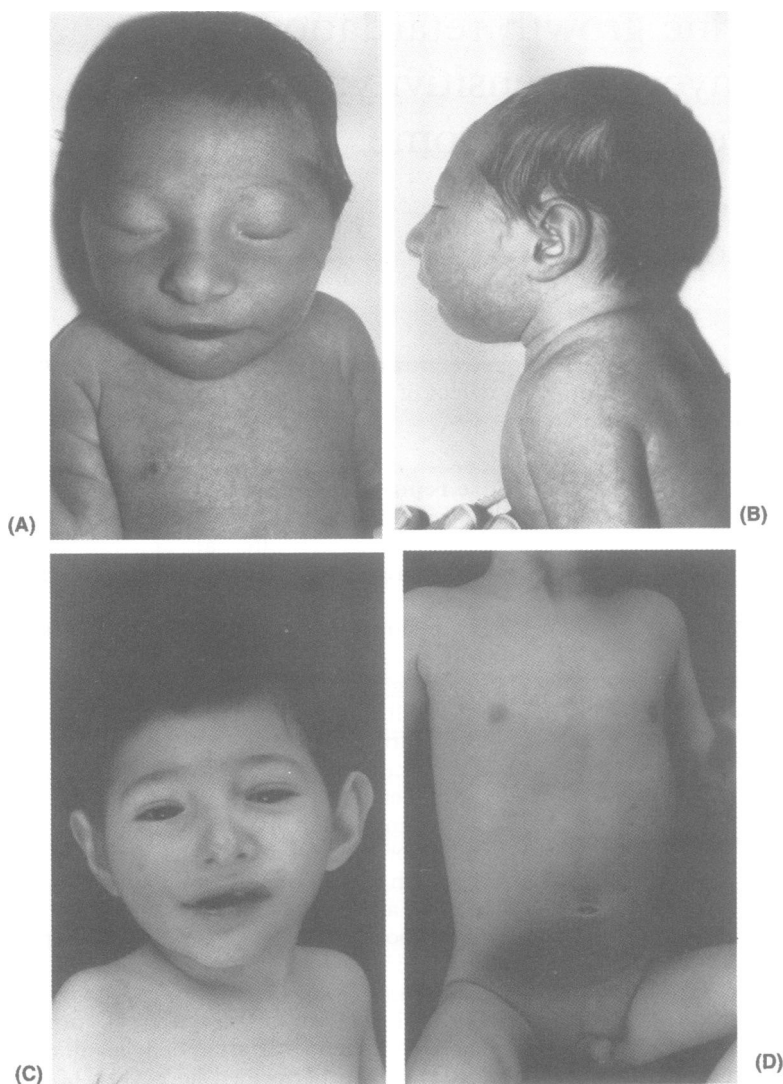


Figure 1 (A) Face at 2 weeks. (B) Side view of head at 2 weeks. (C) Face at 16 months, showing little resemblance to Seckel syndrome. (D) Body at 16 months; scattered small hyper- and hypopigmented skin patches can be seen on the abdomen.

moderately spastic with flexion deformities of the elbows and ankles. He could not sit without support and had a tuneless babbling vocalisation with no discernible words or understanding.

He was admitted to hospital at 16 months for investigation of bleeding from the gums and mouth, a petechial rash, and spontaneous bruising. A full blood count showed pancytopenia, typical results being: Hb 8.3 g/dl, MCV 65.4 fl, MCH 21.3 pg, MCHC 32.5 g/dl, platelets $24 \times 10^9/l$, white cell count $5.1 \times 10^9/l$ with a differential showing occasional myelocytes, 1% metamyelocytes, 8% basophils, 0% neutrophils, 57% leucocytes, 33% monocytes, 1% reactive lymphocytes, and no nucleated red blood cells. A provisional diagnosis of Fanconi anaemia was made but this was excluded by a normal bone marrow examination; bone density and fragment size, cellularity, and trails were normal, as were erythropoiesis, granulopoiesis, megakaryocytes, and lymphocytes, and no neoplastic cells were identified. A poor dietary input and an acute viral illness were thought to be contributing to pancytopenia. In view of

the poor prognosis with or without treatment he was managed with supportive care and died one week later, aged 17 months, with bilateral bronchopneumonia.

At necropsy there were extensive areas of consolidation in both lungs owing to focal pneumonia, moderate bronchiectasis, and bronchiolitis. The left ventricle showed moderate endocardial fibroelastosis. Superficial erosions were seen in the stomach. The thymus was small and involuted. Bone marrow sampled at multiple sites had focal lymphoid infiltration but no hypoplasia. Lymph nodes had normal architecture but only occasional germinal centres. Lymph node sinuses contained large plasmacytoid cells and some macrophages with erythrophagocytosis. Other internal organs showed no abnormalities. At the parents' request the central nervous system was not examined.

Cytogenetic investigation

Chromosome analysis was performed twice, at 9 months and 13 months, on PHA stimulated blood lymphocytes and once at 16 months on fibroblasts established from a skin biopsy. Mitomycin C at a concentration of $0.1 \mu\text{g/ml}$ was added to fibroblast cultures for the 24 hours before harvest. Both chromosome breakage and sister chromatid exchanges (SCEs) were scored. A fibroblast cell line from a child with Fanconi anaemia was included for comparison in the mitomycin C experiment. The SCE rate was determined by the addition of BrdU to cultures for the final 48 hours. To assess sensitivity to ionising irradiation 25 rads of x rays were given six hours before harvest and chromatid and chromosome breaks scored. Standard cytogenetic methodologies were used.⁷

Both fibroblasts and lymphocytes grew normally under standard culture conditions. The karyotype was normal male, 46,XY, in lymphocytes and fibroblasts. The cytogenetic results scoring SCEs, spontaneous and mitomycin C and x ray induced damage are summarised in the table. Multiple spontaneous breaks and non-homologous translocations were found in lymphocyte and fibroblast cultures. In the first lymphocyte sample there were 8.4 breaks per cell (12 cells being examined) and in the second 1.8 breaks per cell (50 cells being examined), the laboratory norm being 0.2 breaks per cell. A number of different unbalanced and balanced translocations were also seen (fig 2). The only anomaly occurring in more than one cell was an inverted chromosome 14 in two of 50 lymphocytes. A very similar increase in mitomycin C induced breakage was seen in our case and Fanconi anaemia fibroblasts. The spontaneous and mitomycin C induced SCEs were comparable to the normal control culture. Ionising irradiation did not cause an increased chromosome or chromatid breakage rate (1.3 v 1.5 breaks per cell, $p=0.6$) as compared to the control culture where an increase was seen (0.1 v 0.6 breaks per cell, $p<0.001$), the results in the patient being significantly different from those seen in the control ($p<0.001$, using 2×2 analysis). The parents' karyotypes were normal

and did not have an increased spontaneous chromosome breakage rate.

Discussion

The patient described here fulfils the clinical diagnostic criteria for a Seckel-like intrauterine dwarfing condition.^{3,4} The facial features, the severity of the microcephaly, and the growth retardation distinguishes Seckel syndrome from other disorders. Clinical, radiological, and cytogenetic findings eliminated other differential diagnoses causing significant microcephaly and growth retardation, such as Bloom syndrome, Cockayne syndrome, chromosome abnormalities, Cornelia de Lange syndrome, Dubowitz syndrome, Seemanova syndrome, and osteodysplastic primordial dwarfism types I, II, and III.

Pancytopenia has been previously described in children with probable Seckel syndrome. Cases 3 and 4 from Seckel's original paper are sibs with birth weights of 1300 g with significant postnatal growth retardation and moderate mental retardation. Both had pancytopenia but did not have the bird headed appearance.¹ Upjohn⁸ reported a family of six children, five of whom were dwarfed and three of these died of respiratory infections. The birth weights were not given but the oldest child, a male, had severe microcephaly and growth retardation, recurrent respiratory infections and bronchiectasis, and developed pancytopenia which led to his death at the age of 12 years. His sister was of similar stature but at the age of 9 was not anaemic. The illustrations in the paper show a Seckel-like face. Butler *et al*⁹ describes a female with a birth weight of 1360 g with significant postnatal growth retardation and

Summary of cytogenetic investigations on fibroblasts

	Control		Case		Fanconi anaemia	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
0.1 µg/ml mitomycin C induced breakage						
No of cells	50	50	45	10	38	9
Breaks per cell	0.1	1.8	0.7	6.9	0.5	5.7
25 rads x ray induced damage						
No of cells scored	50	50	21	27		
Breaks per cell	0.1	0.6	1.3	1.5		
Chromatid breaks per cell	0.1	0.55	1	1.1		
0.1 µg/ml mitomycin C induced sister chromatid exchange						
No of cells scored	58	27	23	24		
SCEs per cell	10.5	21	11	21		



Figure 2 Lymphocytic chromosome preparation showing an increased level of chromosome and chromatid damage (arrow heads).

microcephaly, a Seckel-like face, and moderate mental retardation. She developed pancytopenia at the age of 8 years and died at the age of 12 years. Chromosomal analysis had shown a normal female karyotype, 46,XX, and a 10% chromosome breakage rate which was significantly higher than in controls. The second case described by Butler *et al*⁹ was a male with a birth weight of 1210 g who had frequent respiratory infections in the first few years of life and tonic-clonic seizures starting at 2 years. He had significant postnatal growth retardation, severe microcephaly, mental retardation, bilateral iris colobomas, a left cataract, cryptorchidism, and a right phrenic nerve paralysis. The spontaneous chromosome breakage rate was not stated but there was an approximately three fold increase in aberrations seen after treatment with mitomycin C and a probable increased rate of sister chromatid exchange. Esperou-Bourdeau *et al*¹⁰ reported two patients with some features of Seckel syndrome, severe growth retardation and microcephaly, a Seckel-like facies, and mental deficiency; the first was a female who became pancytopenic at 15 years of age, had a hypoplastic bone marrow, increased spontaneous chromosome breakage, and was successfully treated with bone marrow transplantation; the second was a male who had a pelvic kidney and bilateral (undefined) thumb anomalies but a normal karyotype and response to alkylating agents (making Fanconi anaemia an unlikely diagnosis), who developed pancytopenia at the age of 10 years.

Lilleyman¹¹ described two sisters with a Seckel-like face with birth weights of 1700 and 2200 g and head circumferences of 27.5 and 30 cm. Both showed severe postnatal growth retardation and microcephaly and were of normal intelligence. Sib 1 developed pancytopenia at the age of 5 years and died at the age of 7 weighing 10 kg with a height of 95 cm. Her sister had an accessory right thumb and developed pancytopenia at the age of 4½ years. Both had a hypoplastic bone marrow but normal female karyotypes. The cases described by Lilleyman¹ are more likely to have had Fanconi anaemia than Seckel syndrome as the birth weight is greater than expected in Seckel syndrome and intelligence was normal. The accessory thumb is a radial ray anomaly, and such findings are frequent in Fanconi anaemia but has not been previously reported in Seckel syndrome.

There are now at least seven reported cases of Seckel-like intrauterine growth retardation with pancytopenia^{18,9} and it is therefore likely that the association is real. In Seckel syndrome increased chromosome breakage has been reported only when pancytopenia has occurred. This syndrome seems distinct from other causes of pancytopenia, Fanconi anaemia, non-genetic causes, the WT syndrome, and possibly Dubowitz syndrome.¹² Fanconi anaemia often causes pre- and postnatal growth retardation and microcephaly but not to the same degree as seen in this case or in Seckel syndrome. Also significant mental retardation and spasticity have not been reported in Fanconi anaemia and congenital anomalies are frequent.¹³ We are unsure of the cause of the pancytopenia

seen in the presented case. It may have been secondary to an undiagnosed lymphoreticular malignancy. In similar cases of Seckel syndrome with pancytopenia the marrow histology has not been reported.

Lymphocyte and fibroblast cultures from our patient showed a high spontaneous rate of chromosome breakage and non-homologous translocations. Fibroblasts exhibited an increased sensitivity to mitomycin C that was comparable to a Fanconi anaemia fibroblast line, a normal spontaneous and induced rate of sister chromatid exchange, and a relative resistance to x ray induced damage in the last six hours of culture. Increased spontaneous and induced chromosome breakage has been described in a number of syndromes caused by disorders of DNA repair and replication. The coincidence of finding increased chromosome breakage in our case and that described by Butler *et al*⁹ and Esperou-Bourdeau *et al*¹⁰ makes it likely that this will be a marker of this syndrome.

Chromosome breakage has only previously been specifically sought in one other sib pair with Seckel syndrome. No increased spontaneous breakage was found and the sister chromatid exchange rates were normal.¹⁴ We found no evidence of increased sister chromatid exchange although this was found by Butler *et al*.⁹ A 26 year old female with "Seckel syndrome" and acute myeloid leukaemia has been reported.¹⁵ She responded poorly to cytotoxic therapy, and although chromosome breakage studies were not performed she may also have had "severe intrauterine growth retardation with increased mytomycin-C sensitivity".

Fanconi anaemia is the only previously described human condition exhibiting increased mitomycin C sensitivity. At least four different genotypes are suggested by cell complementation studies, A, B, C, and D. The gene for group C has been cloned but shows no recognisable homology to other genes or proteins.¹⁶ At least 15 distinct non-complementing laboratory generated mitomycin C sensitive cell lines have been described, only one of which may be homologous to Fanconi anaemia.¹⁷ It is probable that the condition described here is a second mitomycin C sensitivity phenotype and is caused by a mutation in one of the genes involved in cross linked DNA strand repair.

As Seckel syndrome may be heterogeneous, we prefer to call this condition "severe intrauterine growth retardation with increased mitomycin C sensitivity". The distinguishing features of this intrauterine growth retardation syndrome are a specific facies together with spontaneous chromosome breakage, increased mitomycin C sensitivity, and later onset pancytopenia. Although the mode of inheritance, and hence recurrence risk, is unclear, prenatal diagnosis may be possible by assessing chromosome breakage. The fibroblast cell line MI-C445 is available from the Murdoch Institute for comparison with defined DNA repair disorders.

1 Seckel HPG. *Bird headed dwarfs*. Basel: Karger, 1960.

2 Harper RG, Orti E, Baker RK. Bird-headed dwarfs (Seckel's syndrome). *J Pediatr* 1967;70:799-804.

- 3 Majewski F, Goecke T. Studies of microcephalic primordial dwarfism. 1. Approach to a delineation of the Seckel syndrome. *Am J Med Genet* 1982;12:7-21.
- 4 Thompson E, Pembrey M. Seckel syndrome: an over diagnosed syndrome! *J Med Genet* 1985;22:192-201.
- 5 Prakash L, Prakash S, Sung P. DNA repair genes and proteins of *Saccharomyces Cerevisiae*. *Annu Rev Genet* 1993; 27:33-70.
- 6 Wevrick R, Buchwald M. DNA repair genes. *Curr Op Genet Devel* 1993;3:470-4.
- 7 Rooney DE, Czepulkowski BH. *Human cytogenetics*. Vols 1 and 2. Oxford: Oxford University Press, 1992.
- 8 Upjohn C. Familial dwarfism associated with microcephaly, mental retardation and anaemia. *Proc Soc Med* 1955;48: 334-5.
- 9 Butler MG, Hall ED, Maclean RN, Luzzio CB. Do some patients with Seckel syndrome have haematological problems and/or chromosome breakage? *Am J Med Genet* 27: 645-9.
- 10 Esperou-Bourdeau H, Leblanc T, Schaison G, Gluckman E. Aplastic anemia associated with "bird-headed" dwarfism (Seckel syndrome). *Nouv Rev Fr Hematol* 1993;35: 99-100.
- 11 Lilleyman JS. Constitutional hypoplastic anaemia associated with familial "bird-headed" dwarfism (Seckel syndrome). *Am J Pediatr Hematol* 1984;6:207-9.
- 12 Berthold F, Fuhrmann W, Lampert F. Fatal aplastic anaemia in a child with features of Dubowitz syndrome. *Eur J Pediatr* 1987;146:605-7.
- 13 Kwee ML, Kuyt LP. Fanconi's anaemia in the Netherlands. In: Schroeder-Kurth TM, Auerbach AD, eds *Fanconi's anaemia*. Berlin: Springer Verlag, 1989:18-33.
- 14 Cervenka J, Tsuchiya H, Ishiki T, Susuki M, Mori H. Seckel's dwarfism: analysis of chromosome breakage and sister chromatid exchanges. *Am J Dis Child* 1979;133: 555-6.
- 15 Hayani A, Suarez CR, Molnar Z, LeBeau M, Godwin J. Acute myeloid leukaemia in a patient with Seckel syndrome. *J Med Genet* 1994;31:148-9.
- 16 Strathdee CA, Gavish H, Shannon WR, Buchwald M. Cloning of cDNA's for Fanconi's anaemia by functional complementation. *Nature* 1992;356:763-7.
- 17 Collins AR. Mutant rodent cell lines sensitive to ultraviolet light, ionizing radiation and cross-linking agents: a comprehensive survey of genetic and biochemical characteristics. *Mutat Res, DNA Repair* 1993;293:99-118.